



## **NOAA Technical Memorandum NMFS**

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# **INVESTIGATION OF THE POTENTIAL INFLUENCE OF FISHERY-INDUCED STRESS ON DOLPHINS IN THE EASTERN TROPICAL PACIFIC OCEAN: RESEARCH PLANNING**

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## ABSTRACT

There is concern that fishing methods used in the eastern tropical Pacific (ETP) tuna purse-seine fishery may cause physiological stress to the dolphin species involved. A workshop to discuss potential methods of measuring fishery-induced stress on dolphins in the ETP was held by the Southwest Fisheries Science Center in La Jolla, California on July 9-11, 1997. The primary goal of the workshop was to identify physiological and morphological indices that can be used to reliably indicate the effects of stress in free ranging dolphins involved in the tuna fishery. Background information presented at the workshop is included in this report as a description of the main characteristics of the tuna dolphin fishery and the dolphin species involved, and a discussion of what is known about the current status of dolphin stocks. Some logistical considerations regarding sample collection from dolphins involved in the tuna purse-seine fishery are included. We introduce the concept of stress and our ability to identify and measure stress in mammals, and briefly review recent advances in stress research. We also summarize participant presentations on physiological stress and clinical indices of stress in cetaceans and domestic animals. Three types of investigations that may provide data on the physiological stress response in dolphins involved in the tuna purse-seine fishery are outlined. These include: 1) a Necropsy Program to examine tissues from dolphins killed during fishing operations, 2) a Repeated Chase/Capture study designed to analyze blood chemistry of samples collected from live dolphins during a regime of repeated chase and encirclement, and 3) Historical Analysis examining specimens and data previously collected from dolphins involved in the tuna purse-seine fishery.

## INTRODUCTION

The Declaration of Panama, a 1995 agreement negotiated between the United States and eleven other fishing nations, is one of several recent efforts to reduce dolphin mortality in the eastern tropical Pacific Ocean (ETP) tuna purse-seine fishery. The agreement imposes a total mortality limit of 5,000 dolphins per year, and intends that all countries will take steps to eliminate mortality entirely. The International Dolphin Conservation Program (IDCP) Act, a 1997 amendment to the Marine Mammal Protection Act, was created to implement the agreement by allowing the importation of currently embargoed yellowfin tuna into the United States. The law includes provisions that could allow tuna caught by the intentional encirclement of dolphins with a purse-seine net in the ETP to be declared "dolphin safe" if no dolphins are observed to be killed or seriously injured in that set.

There is concern, however, that fishing methods used by the ETP tuna-dolphin purse-seine fishery may cause stress to dolphins, and that such stress may have an adverse impact on reproduction or survival of these animals. As a result, the IDCP Act requires that research, consisting of population abundance surveys (see Gerrodette et al., 1998) and "stress studies," be conducted by the National Marine Fisheries Service (NMFS) to determine whether the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock." Initial findings, based on preliminary results of the research program, are to be made by March of 1999, with final conclusions and recommendations reported to congress by the year 2002.

To begin planning for this research, a workshop to discuss potential methods of measuring fishery-induced stress on dolphins in the ETP was held by the Southwest Fisheries Science Center (SWFSC) in La Jolla, California on July 9-11, 1997. The main objectives of the workshop were to summarize the current state of research on stress in non-human mammals and to determine the most appropriate techniques to investigate the potential for fishery-induced physiological stress response in ETP dolphins. Our specific goals were to identify physiological and morphological indices that can be practically measured to quantify the effects of stress in these dolphins. Participants in the workshop included veterinarians specializing in marine mammals, as well as experts on stress physiology, energetics, pathology, reproductive endocrinology, and the ETP tuna purse-seine fishery (Appendix 1).

This report summarizes recommendations of the workshop participants and contains preliminary plans for three specific research projects.

## ARRANGEMENTS FOR THE WORKSHOP

Edwards chaired the workshop, which was convened by Edwards and Curry. The agenda appears in Appendix 2, background papers are listed in Appendix 3.

Several of the participants gave presentations during the meeting (see Appendix 2), and ensuing discussions were, in part, facilitated by a document outlining detailed questions relevant to the objectives of the workshop (Appendix 4).

## BACKGROUND

Background information was presented at the workshop to familiarize participants with general characteristics of the ETP purse-seine fishery. Discussion of background material allowed participants to assess the opportunities and constraints inherent to designing an investigation of the potential for fishery-induced stress on the populations involved. The history of the tuna-dolphin fishery and general points regarding fishing methods were briefly reviewed.

The tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific for nearly five decades. The association occurs frequently enough that sightings of these dolphins are used by fishermen as a signal that tuna may be present, and purse-seine nets are often set on dolphin schools as a means of catching tuna (Perrin, 1969). Pantropical spotted, *Stenella attenuata*, spinner, *S. longirostris*, and common, *Delphinus delphis*, dolphins are the species most often found in association with tuna (predominately yellowfin, *Thunnus albacares*; Hammond, 1981; Au and Pitman, 1988). The reason for the bond, and the relative importance of it, for either dolphins or tuna, is not known (Hammond, 1981; but see Au and Pitman, 1986; Au 1991; Edwards, 1992).

### Dolphins Involved in the ETP Tuna Purse-Seine Fishery

Edwards presented background information on the primary dolphin species and stocks involved in the ETP tuna fishery (see Perrin *et al.*, 1985; Perrin *et al.*, 1991; Dizon, *et al.*, 1994). Of the dolphin species most frequently targeted (pantropical spotted, spinner, and common dolphins) three major stocks are commonly affected. These are the northeastern

spotted dolphin, and the eastern and whitebelly spinner dolphin stocks (Smith, 1983; Wade and Gerrodette, 1993; Wade, 1995). The northeastern spotted dolphin is the target of approximately 85% of all sets on dolphins. Spotted dolphins occur mostly in single-species schools, but a significant fraction of schools also include eastern spinner dolphins.

#### *Estimated Mortality*

Historically, levels of dolphin mortality in purse-seine nets were extremely high (Smith, 1983; Lo and Smith, 1986; Wade, 1995). Wade (1995) estimated that 4.9 million dolphins were killed by the purse-seine fishery over a fourteen year period (1959-1972). Mortality decreased gradually because of measures such as the Marine Mammal Protection Act (1972) and use of equipment designed to prevent dolphin entanglement in purse-seine nets (Barham *et al.*, 1977). Despite these changes, dolphin mortality continued to occur at levels that were probably higher than the stocks impacted by the fishery could sustain (Wade, 1995).

Since 1992, dolphin mortality in the fishery has significantly decreased to a few thousand animals per year (Hall and Lennert, 1993, 1994, 1997; Lennert and Hall, 1995, 1996). A number of legislative measures, including US embargoes and the advent of the "dolphin-safe" policy, caused changes in the fishery and mortality was greatly decreased (see Joseph, 1994). Wade (1995) concluded that these low levels of mortality could allow recovery and growth of the impacted stocks. Total observed mortality for 1996 was 2,547 dolphins (Lennert and Hall, 1997) and the preliminary estimate for 1997 is approximately 3,000 dolphins (Inter-American Tropical Tuna Association, IATTC, 1997).

#### *Status of Dolphin Stocks*

Gerrodette provided information on the population status of pantropical spotted and spinner dolphins in the ETP (see Wade and Gerrodette, 1993; Wade, 1993). Wade and Gerrodette (1993) estimated 730,000 northeastern offshore spotted dolphins, 630,000 eastern spinner dolphins, and 1,000,000 whitebelly spinner dolphins in the ETP. Wade (1993, 1994) determined that the offshore spotted and eastern spinner dolphin stocks were depleted as defined by the Marine Mammal Protection Act. Since 1975, the abundances of offshore spotted and eastern spinner dolphin stocks have apparently been relatively stable. Since about 1992, the abundance of these stocks should be increasing because of the low mortality in the purse-seine fishery. Gerrodette noted that the current

observed fishery mortality should have a negligible effect in terms of population dynamics. However, Gerrodette cautioned that Wade's (1994; and see Wade, 1998) model estimating population size of these dolphins was based upon perturbation from mortality and population density, but does not estimate possible effects of fishery induced stress.

#### ETP Tuna Purse-Seine Fishery

Edwards reviewed specific elements of the methods involved in tuna-dolphin purse-seining. The fishery targets yellowfin and skipjack (*Katsuwonus pelamis*; 4% of annual catch) tuna. There are three types of sets: 1) dolphin sets, 2) schoolfish sets, and 3) log sets. The yellowfin tuna known to associate with dolphins are typically 2-4 years old and about 80-125 cm total length. Tuna caught in schoolfish and log sets are generally smaller.

#### *Oceanographic Location and Seasonal Influences*

The yellowfin fishery operates along the coast of Central and South America from Baja California to approximately 20°N, and seaward to approximately 150°W, south of Hawaii (Figure 1).

Although there is fishing effort throughout the year, there is significant spatial, seasonal, and interannual variability of oceanographic conditions (Reilly, 1994). Distribution of tuna and dolphins, and amount of tuna catch, create differences in effort among regions within the fishing grounds.

Scott introduced a discussion of the use of life history parameters to assess changes in population status due to fishery exploitation. Chivers and Myrick (1993) examined life history parameters for two exploited spotted dolphin stocks and found no evidence of expected compensatory changes in response to decreases in abundance. Chivers and DeMaster (1994), however, used a larger data set to investigate life history parameters for three dolphin species (*S. attenuata*, *S. longirostris*, and *D. delphis*) in the ETP and found that compensatory mechanisms did appear to be operating in response to changes in population density. Chivers and DeMaster (1994) concluded that life history data can be used as biological indices to distinguish between a population responding to change in abundance due to fishery exploitation, and changes in carrying capacity when relationships between life history parameters and population status are known. Investigation of life history parameters for ETP dolphin stocks may provide information regarding the relative effects of physiological stress and mortality in these populations. Scott suggested that it may be necessary to include estimates of fishing effort to accurately assess the biological impact

on populations involved. It must be noted that in order to detect changes in life history parameters, large sample sizes will be required.

### *Composition of Fleet*

During the late 1990's, the purse-seine fleet in the ETP has included approximately 100-120 vessels. About 50% have been Mexican vessels, the remainder have been from a number of other nations (about six US vessels currently participate in the fleet; under US law it is illegal for these vessels to fish on dolphins).

### *Observer Program*

In 1972 the NMFS began a tuna/porpoise observer program to monitor US vessels by collecting data on dolphins and their association with the ETP fishery. In 1979, the IATTC initiated a similar observer program for the international fleet. By 1995 the IATTC had assumed full responsibility for placing observers on all US vessels. Observers are trained to record biological data for all phases of the seining operations. Observer data forms are shown in Appendix 5.

### *Characteristics of Fishing Vessels, Operation and Gear*

The Purse-Seining Manual (Ben-Yami, 1994), contains detailed information regarding purse-seines, auxiliary gear, and operation. Purse-seines are rectangular nets (approximately 1.6 km long by 200 m deep) constructed of synthetic materials, and can weigh up to several tons. Vessels involved in the tuna purse-seine fishery are generally 50 to 75 meters long and capable of storing 400-1,700 tons of whole, frozen tuna for months. Fish are stored in large tanks of brine, below decks. Most of the more recently built vessels (after 1970) have carrying capacities (tuna catch) of 1,000-1,400 tons. Vessels require a crew of 15 to 24 men.

Two or three diesel generators provide power to start the main engine(s), run the refrigeration system, and operate electricity. Most boats are equipped with sophisticated navigation and communication equipment and short-range fish-finders.

### *Fishing On Dolphins*

Edwards described fishing methods beginning with the search. Fishermen use 25power and/or hand-held binoculars from the bridge or mast lookout, S-band "bird-radar," and X-band radar to detect cues. A helicopter is used to search for dolphin schools and to assess whether or not a school carries sufficient tuna to make a set. When a dolphin school is located, the seiner



approaches traveling at approximately 15 knots. Dolphins are reported to react to the vessels from a distance of approximately eight kilometers.

Four to six speedboats (85-140 hp) are used to chase the school, and to separate and herd a portion of the school to be encircled. The helicopter may also be used to herd animals during these operations, which is said to last from 20-40 minutes but can sometimes take more than two hours.

The purse-seine net is set with the aid of a skiff deployed from the net stack at the rear of the vessel. The skiff acts as an anchor for one end of the net as the seiner moves away in a circle, setting the net continuously behind it. Speedboats herd the dolphins into the closing arc of the purse-seine (dolphins sometimes escape by running across the narrowing opening as the net is pulled into a circle, or diving under the sinking net). Dolphins tend to gather in the center of the net as it is pursed at the bottom. Backdown, a procedure for releasing dolphins over the net's corkline, begins after about two-thirds of the net has been hauled aboard. A channel is formed at the far side of the net and the corkline is submerged so that the dolphins can exit. Speedboats may be used to pull the corkline, thus keeping the channel from collapsing. Crewmen enter the water, when necessary, to pull dolphins over the corkline. Encirclement takes approximately 40 minutes, and dolphins may be confined for about an additional hour (longer if unanticipated problems occur).

## Capture Rate

Dividing the number of dolphins in the ETP by the number of sets annually gives a simplistic capture rate of eight captures/animal/year. An analysis considering the fact that tuna fishermen actively search for, and set on, larger schools (and based on the assumption that individual dolphins choose to remain in schools of a particular size), indicates that about 10% of the northeastern spotted dolphin stock may be set on as often as once per week (Perkins and Edwards, 1997). Approximately 30% of the stock may be set on 2-8 times per year, and about 50% of the stock may be set on only once or twice a year. The analysis, however, is complicated by the fact that dolphin herds are not stable entities and may fluctuate in size as often as on a daily basis.

### *Behavior*

There is some indication that dolphin behavior has changed since the early years of the fishery. Some information suggests that dolphins in areas with high historical fishing effort are more experienced at avoiding chase and encirclement than more naive dolphins in areas where fishing had expanded more recently. Fishery observers have noted that dolphins apparently anticipate backdown because they move to the backdown channel and "line-up" for release.

Dolphins have been observed being hyperactive as well as passive, when confined in purse-seine nets. Several passive behaviors (sleeping, sinking, and rafting) have been described for dolphins encircled in purse-seine nets, and it has been suggested that such behaviors may have resulted from stress (Norris et al., 1978; Coe and Stuntz, 1980). Scott noted that dolphin behavior in the nets appears to have changed over the years, and that some behaviors are not seen as commonly. There is anecdotal information that "sleeping" behavior, on the net bottom, has become rare. Rafting behavior is still often observed, and has been suggested to be a learned behavior for avoiding net-entanglement, and has also been hypothesized to be induced by stress (Coe and Stuntz, 1980; Sevenberg, 1997). The significance and current prevalence of these behaviors is not fully known.

## PRACTICAL ASPECTS OF SAMPLE COLLECTION

Scott discussed logistical aspects of sampling and experimental opportunities for studying ETP dolphins.

### Captive Dolphins

Scott suggested that oceanaria or research facilities can provide the necessary environment to allow comparisons between experimental and control animals. Consequently, sophisticated methodologies and rapid laboratory analyses might be more feasible in these settings, than for work at sea. Unfortunately, the only available captive animals are most often species other than those most commonly involved in the fishery (bottlenose dolphins, *Tursiops truncatus*; occasionally live-stranded Atlantic spotted dolphins, *S. frontalis*).

<sup>4</sup> A study regarding "evasive behavior" of spotted dolphins was completed by G. Heckel, Universidad Autonoma Baja California, Baja California, Mexico, and has been summarized in the NMFS Tuna Newsletter, November 1997.

At the present time, there is one female pantropical spotted dolphin at Gulfarium, Florida (captive since 1993), and one male Atlantic spotted dolphin at the Dolphin Research Center, Florida (captive since 1986).

#### Post-Mortem Specimens

There are many samples available from dolphins that have been killed in the fishery over the past twenty-five years. The SWFSC has a large collection of teeth and reproductive organs, as well as skin and blubber tissues (Table 1). In addition, fetuses, stomach contents, adrenal glands, and other tissues have been collected.

Scott noted that observers could begin collecting samples, or whole carcasses, if specimens are required from current fishery kills. Approximately 2,500 - 3,000 animals are now killed per year (700 northeastern spotted dolphins). Not all of these will be available for study, but potentially several hundred samples per year could be collected. This work would require both domestic and international permits.

#### Live-Capture of Free-Ranging Dolphins

Free-ranging dolphins have often safely been captured while riding the bow of a ship (using a hoopnet or tailgrab). Capture could allow blood samples, heart rate, ultrasound information, and tagging of individuals prior to release. Scott suggested that a comparison between spotted dolphins in Hawaii and those in the ETP might be useful to investigate physical differences between populations that are set on and those that do not experience that type of fishery interaction.

Two successful spotted dolphin purse-seine live-capture/tagging operations were conducted in 1992 and 1993 to track tuna and dolphins in the ETP (Armstrong, 1993, 1994; IATTC, 1993). Dolphins encircled by purse-seine nets were caught by swimmers just prior to backdown. Investigators were able to capture individuals, place them in a raft, collect blood samples, attach a radio transmitter, and release the animal in 7-12 minutes.

It was also noted that biopsy samples are routinely taken, using retrievable biopsy darts, on NMFS marine mammal research cruises. Such samples may be useful for determining cortisol levels in these dolphins (see Research Planning and Appendix 6), as well as for genetic, fatty acid, and isotope analyses. In the ETP, however, biopsies are not always easily taken because many schools actively avoid ships.

## STRESS IN MAMMALS

Subsequent to discussion of background information, the concept of stress and our ability to identify and measure stress in animals was introduced. Animal stress is difficult to identify and measure, in part, because there is no single consistent biological response to stress (Levine, 1985; Moberg, 1987; Sapolsky, 1994). There have been, however, many recent advancements in stress research. Specifically, many studies have contributed to our ability to measure hormonal interactions and responses to stress (Axlerod and Reisine, 1984; Levine, 1985; Breazile, 1988; Chrousos and Gold, 1992), the effects of stress on suppression of immune function (Keller *et al.*, 1991; Mal *et al.*, 1991), and disruption of reproduction (Moberg, 1991; Rivier and Rivest, 1991). Several recent studies have also been able to characterize and quantify the effects of pursuit or chase on animals (Harlow *et al.*, 1992; Bleich *et al.*, 1994; Bateson and Bradshaw, 1997; see Bateson, 1997).

In an effort to provide a clinical means of measuring of stress in animals, Moberg (1987, 1992) suggested that the most reliable indicator may be the prepathologic state resulting from the stress itself. Based in part on Moberg's work, Dierauf (1990b) provided a preliminary list of clinical indices of stress in marine mammals. Many of the conditions noted by Dierauf (1990b), however, were based on evaluations of captive animals.

Three specialists participating in the workshop gave presentations organized to delineate reliable indicators of stress in the free-ranging dolphins involved in the ETP tuna fishery. Cowan's presentation, based on his extensive research of comparative pathology including human, terrestrial and marine mammal studies, discussed the concept of stress and the potential for measuring stress using animal tissue collected during necropsy. Geraci presented information based on his experience studying stress in marine mammals, and emphasized the known clinical indices of stress in cetaceans. Friend presented results of his research on behavioral and physiological stress in domestic animals and discussed ways in which his methodologies could be applied to dolphins.

### The Meaning, Mechanisms, and Consequences of Stress

Cowan discussed the concept of stress, describing it as the demand for adaptation to a changing environment. He noted that stressors, which can be defined as any perturbation that threatens an organism's internal homeostasis, incur bioenergetic costs to the individual. Cowan emphasized that, in some circumstances, the extent and nature of the stress response can be

influenced by the animal's perception of the stressor (i.e. there is a psychological component to stress). In general, modifications of behavior, activation of the autonomic nervous system, and changes in the neuroendocrine system may all be incorporated into the body's response to stressors (see Moberg, 1985, 1987; Figure 2). The stress response results in the mobilization of energy, the disruption of long-term processes (digestion, reproduction, growth, immune response), diminished sensitivity to pain, and a heightening of the senses (Sapolsky, 1994)

The stress response can range from generalized to acute or chronic, and may eventually become exhaustive. The potential effects, at each of these phases, can vary among individuals. Cowan explained that a generalized stress response, resulting in increased levels of cortisol and other corticosteroids, should be evident in structural changes to the adrenal cortex. An acute stress response may be manifested in autonomic effects such as changes in blood pressure and heartrate, and difficulties in refining temperature control (shivering). A chronic stress response (potentially reaching the level of adrenal exhaustion) involves foregoing costly energetic functions to maintaining basic life processes and can result in reproductive failure, abnormal growth, and immune suppression.

Cowan particularly noted that there appear to be morphological differences between the relative sizes of the delphinid adrenal cortex and medulla in comparison to other mammals. The medulla is very large in dolphins, and Cowan suggested this may be a structural adaptation for responding to the physiological demands of the marine environment. Comparing the weight of the adrenal cortex to the medulla could show response differences between these dolphins and other dolphin populations or even other mammals.

Cowan also emphasized that histological examination of tissues from stranded animals and fisheries kills has indicated chronic stress may cause damage to the heart muscle and vasculature. These ischemic injuries may be caused by long-term exposure to high levels of adrenaline, and could provide an index or an indication of fishery-induced stress in ETP dolphins (see Cowan and Walker, 1979; Cowan et al., 1986).

Building upon the information given during Cowan's presentation, a plan was developed for a proposed necropsy sampling program of dolphins killed in the ETP fishery. This program is discussed in the Research Planning section of this report.

## Physiological Stress Response in Cetaceans

Geraci reviewed what is known about stress in cetaceans, presenting examples of clinical measures of stress for several species (see Geraci and Medway, 1973; Geraci and St. Aubin, 1979; Thompson and Geraci, 1986; St. Aubin and Geraci, 1988; Dierauf, 1990b; also see Suzuki *et al.*, 1998). Geraci noted that in adapting to a wide range of conditions in the marine environment, cetaceans have developed some possibly unique features in their physiological response to environmental stressors.

As is common for all mammals, the physiological stress response of cetaceans includes secretion of cortisol from the adrenal cortex to increase lipid and carbohydrate metabolism and promote the breakdown of proteins. Cortisol also reduces the inflammatory response. Geraci said that certain biochemical characteristics of the cetacean physiological stress response appear to differ from those of other mammals. For example, cortisol levels in cetaceans responding to stress appear to be modest in comparison to other mammals (Thompson and Geraci, 1986). Aldosterone, which does not characterize the stress response in terrestrial mammals, is released from the adrenal cortex as a response to stress in cetaceans.

Geraci emphasized that blood chemistry can provide clinical indices of chronic exposure to stress in cetaceans. For example, blood sodium concentration, reduced blood eosinophil concentration, and reduced serum iron levels can be reliable indicators of stress. Other clinical features stress, leading to shock, in cetaceans may include hypoperfusion of the muscle, liver, and other abdominal organs, intravascular coagulation, tissue necrosis, and hypoxia.

Geraci commented that rafting observed in netted dolphins may not be a healthy behavior. He mentioned that harp seals, *Phoca groenlandica*, are known to go into catatonia in initial response to a novel stressor. Geraci also noted that bradycardia and apnea appear to be associated with fear in some phocid seals and might be a part of the clinical profile of dolphins affected by the tuna fishery. He speculated that these dolphins may, with time and experience of being chased and captured, accommodate and be able to avoid that type of parasympathetic stress response.

Building upon information given during Geraci's presentation, plans were developed for a proposed sampling program involving repeated chase and capture of dolphins involved in the ETP tuna purse-seine fishery. The program is described in the Research Planning section of this report.

## Behavioral and Physiological Stress Response in Domestic Animals

Friend presented a brief history of the study of stress and the stress response. He discussed Selye's (1950) theory of a general adaptation syndrome, noting that the mammalian body has a similar set of responses to a wide variety of stressors. Selye emphasized the role of the adrenal cortex in releasing cortisol under stressful conditions. According to Selye's model, after the initial alarm phase, there is a short-term release of cortisol and after a period of "shock," there may be a low level of cortisol release. Beyond these phases of the stress response, an extreme stressor might lead to an adrenal exhaustion phase.

Friend also remarked on the psychological component of stress, saying that it can play an important role in the degree of response and can lead to variation among individuals. In his own research, Friend has found that an animal's perception of the degree of control it has over a situation will significantly affect the stress response (Friend, 1991). In addition, Friend said that because dolphins are herding animals, like horses and cattle, social separation, such as may occur during chase and encirclement, will likely be stressful to them.

In discussing his research (Friend, 1991; Friend et al., 1988, 1996; Lanier et al., 1995), Friend underscored the importance of integrating knowledge obtained from studying terrestrial mammals into studies of dolphins involved in the ETP fishery. Friend discussed several studies in which domestic animals were separated and maintained in a number of ways, and the differences that were seen in physiological responses to separation and confinement. Friend outlined the methods he has used to measure physiological responses to stress in domestic animals including analysis of blood samples for circulating hormone levels (particularly cortisol) and immunological components, examination of adrenal gland function and morphology, examination of thyroid gland function, and monitoring heart rates. Friend concluded that these measures would likely prove useful in dolphins if the practical problems of measurement could be overcome.

## RESEARCH PLANNING

After initial presentation of background material and relevant information on physiological stress in cetaceans, participants focused on discussing potential research projects that could address the question of whether fishery-induced stress may be having a significant adverse impact on dolphins in the ETP. These discussions led to agreement that three types of investigations may

provide useful data: 1) a Necropsy Program to examine tissues from dolphins killed during fishing operations, 2) a Repeated Chase/Capture study designed to analyze blood chemistry of samples collected from live dolphins during a regime of repeated chase and encirclement, 3) Historical Analysis examining specimens and data previously collected from dolphins involved in the tuna purse-seine fishery. The proposed programs are described in general below.

## Necropsy Program

### *Objectives*

The necropsy program proposes to evaluate the pathophysiological condition of post-mortem specimens. Examination of dead dolphins will provide the opportunity to look for morphological indications of physiological stress. Information from postmortem specimens will also provide insight into the natural states of health and disease for these populations of dolphins in the ETP.

Careful collection and examination of post-mortem specimens will allow histological observation of sublethal effects of fisheries activities on the heart and heart muscle, adrenal glands, the liver and other major organs. The time sequence of injurious events is important and histological evidence of healing, if present, could provide some indication whether observed lesions are several days old or have occurred even more recently.

Relatively fresh blood samples can be obtained from post-mortem specimens to perform complete blood panels, immunological analyses and cell tissue cultures (see Table 2 for some established "normal ranges" in selected blood values for bottlenose dolphins). While information from these analyses may be difficult to interpret, they will at least provide some baseline information for the population.

It is possible that recent estimates regarding frequency of purse-seine capture per individual dolphin (Perkins and Edwards, 1997) can be incorporated into data analyses to arrive at a better understanding of potential effects of capture on the population. Estimating the ages of individuals (Myrick *et al.*, 1983) will be useful for this purpose as well as for use as general life history data.

### *Sample Collection*

The types of samples proposed for collection are indicated in Table 3. Specimens will have to be collected in a rigorous and systematic manner that is not possible for tuna vessel observers under current circumstances (although certain observer-collected specimens may be used to



augment specimens collected for the necropsy program). It will be necessary to have trained technicians or graduate students with some background, or program of study, in pathology to collect samples from specimens. To optimize the amount of information gathered from any single animal, multiple samples must be collected and the procedures could take many hours for a single postmortem specimen.

Cowan emphasized that weighing all organs provides valuable information. In particular the adrenal gland weight could be very important. Microanatomical examination of all organs is essential, and as noted previously, the heart in particular may provide critical information.

Jensen noted the importance of having baseline information on the natural states of health and disease in these dolphin populations. Collected specimens should also be used to provide an overall health assessment. Such information is necessary for deriving conclusions regarding the effects of stress on the animals. Worthy agreed and added that data on body composition and blubber depth will be useful for assessing the general health and nutritional condition of these dolphins.

Czekala added that examination of gonadal tissues can provide basic life history and health information, as well as, indications of compensation for stress.

Palmer presented a method for detecting corticosteroid levels in cetacean blubber samples (Appendix 6) as a means of investigating the stress response in these animals. Geraci indicated that it would be best to obtain baseline information on all steroids in cetacean blubber. Friend agreed and suggested that it would be interesting to look at how all the steroids interact simultaneously. Because we do not know the dynamics of steroids in the blubber, Cowan suggested that it would be useful to assess the metabolic turnover rate by using an exogenous (labeled) steroid in a captive dolphin and measuring diffusion time. Worthy noted that it will also be necessary to know what blubber layer lipids have been extracted from, and the anatomical position from which blubber was sampled.

Curry commented that the chase may be a significant part of the stress on these dolphins, and that the length of the chase may be a factor in the stress response. Geraci noted that there may also be a psychological aspect of the chase affecting the stress response, and that overheated, over exerted muscle does not heal well. Muscle fiber typing, testing for lactic acid and creatine kinase levels, and measuring blood gases may be important for assessment of the degree and effects of muscle and respiratory exertion.

A detailed planning report containing information on major courses of investigation proposed to quantify the effects of stress in dolphins, necropsy procedures,

sampling protocols, and strategies for accommodating logistical constraints will be available as the necropsy research is implemented.

## Repeated Chase/Capture Study

### *Objectives*

The repeated chase/capture study proposes to provide an accurate documentation of the physiological response to incremental levels of speedboat chase and purse-seine capture using analysis of blood constituents for samples collected from live dolphins that have been subjected to these events. A series of measurements of complete blood panels from a subset of captured dolphins can be used to quantify the effects of repeated chase and capture, and may give an indication of the trend of responses among a number of individuals with repeated exposure over time. In addition, repeated sampling will provide a profile of the range of possible conditions among these animals.

The idea that dolphins could be captured and maintained in a large sea-pen was introduced. This type of temporary captivity would allow the collection and analysis of blood samples to examine changes in blood constituents over time, but would not mimic the conditions of repeated chase and capture. In addition, blood sampling of dolphins in the purse-seine net will provide an opportunity to collect blood from both rafting and non-rafting dolphins.

Purse-seine capture will make it possible to study ETP dolphins while they are being affected, and to examine short-term responses. General protocols will be developed such that dolphins encircled in a tuna purse-seine net will be held by swimmers, placed in a raft for sample collection, roto-tagged and some will be fitted with a radio-transmitter. Handling would most likely occur just prior to backdown (after having been chased for approximately 20 minutes and having spent about one hour encircled by the net). The dolphins would then be released and followed (with the aid of radio-transmitters), and recaptured after a period of one or more days.

Geraci noted that blood analysis is advantageous because biochemical changes to the body almost always precede morphological changes. He gave the example that a blood screen for creatine kinase might indicate an elevated level as evidence of some muscle leakage and possibly muscle damage. Blood samples can also be used to indicate the range of organs affected (see Jain, 1993). In addition, a fresh blood sample can provide immunological data. Czekala suggested that blood samples will be useful for assessing female reproductive status. Blood samples can be used to detect pregnancy, and potentially could show effects of chase on hormone levels of pregnant females

(or could provide evidence of abortion during chase or encirclement).

During discussions of the potential for a chase and capture operation, Geraci tabulated predicted changes that might occur in some blood values for dolphins repeatedly captured over time (Table 4).

Geraci also stated that there is enough existing data on normal ranges of baseline cetacean blood values, for reasonable comparisons (see Table 2; see also Medway and Geraci, 1964, 1965; Engelhardt, 1979; Geraci and St Aubin, 1979; Asper et al., 1990; Bossart and Dierauf, 1990; Suzuki et al., 1998).

### Sample Collection

Upon initial capture, a blood sample will be collected and the individual will be roto-tagged for identification. A sub-set of individuals will be fitted with a radiotransmitter so that movement and diving patterns can be monitored. Blood samples (approximately 50 cc) will be collected for each of the roto-tagged dolphins, as is possible. Blood samples can quickly and easily be collected by experienced personnel. Dolphins will be released and, as possible, recaptured up to four times. Sampling and tagging is anticipated to take approximately 10 minutes per individual.

### *terminal sampling*

One difficulty with designing experimental procedures to examine the physiological stress response in ETP dolphin species, is the lack of a satisfactory control group for comparison of potentially stressed and non-stressed individuals. There is not an unaffected, control population for comparisons. During the workshop, several participants agreed that terminal sampling of individuals during different stages of a repeated chase and capture study would provide controls for making definite conclusions regarding the effects of the purse-seine operations. Although intentionally killing individual dolphins would likely provide definitive control samples, it was not considered to be an acceptable option and was not be considered further.

### *blood*

Collection of blood samples will allow analysis of complete panels (including standard blood chemistry, hormonal measurements, hematological parameters) for all animals possible (see Table 2). In addition, current cell culture techniques may allow useful measurements of immune

function and disease resistance. If possible, blood gases will also be measured as an indication of the effects, if any, of forced exercise.

#### *other potential samples and measurements*

Workshop participants suggested additional measurements that may provide useful data regarding the effects of chase and encirclement on dolphins. These included monitoring heartrate and completing electrocardiograms for assessment of general cardiac response to chase, capture, and handling. Worthy noted that assessment of body temperature such as rectal temperature gradient and blubber/muscle temperature gradients would be useful means of examining responses to capture activities.

As measures of general health status, several samples and measurements may be feasible. Culture swabs for microbiological studies (bacteria and viruses) can easily be taken from the blowhole, eyes, genital area, rectum, and any lesions present (Geraci and Lounsbury, 1993; Carter and Cole, 1990). Ultrasonic measurements can be used to measure blubber layer thickness as an index of body condition and to assess pregnancy.

#### *Logistics*

Previous capture operations have provided insight as to the type of vessels, personnel and equipment that will be needed. Extensive pre-cruise practice and testing of research procedures, in addition to prioritizing sample collection, will improve efficiency of the investigation. Only very preliminary logistical requirements are outlined below.

Tagging and capture operations will require the use of a purse-seine vessel so that captures take place under similar conditions to those of the fishery. Use of a research vessel will allow tracking, laboratory work, data analysis, housing of personnel, etc. In addition to transit time, approximately 30 days of ship time during calm seas will be required.

Scientific personnel will include a minimum of the following: One or more veterinarians extensively experienced with the capture, care and handling of marine mammals (veterinary technicians may also be needed), scientists experienced with live-capture operations (particularly with capture by purse-seine of these dolphins in the ETP), scientists experienced with radio/satellite tracking, and marine mammal observers experienced in sighting and identifying dolphins with high-powered binoculars.

Specific types of equipment for animal health assessment (electrocardiograph, ultrasound, etc.) will be required in addition to some shipboard analytical and storage equipment. Underwater

video equipment will be used for recording and observations of dolphin behavior in the net.

### Analysis of Historical Specimens and Data

Workshop participants discussed the scientific value of the numerous specimens collected from dolphins killed in the ETP tuna purse-seine fishery (see Table 1). Many of these samples have already been examined, and have been used to investigate the biology of the populations involved in the fishery (Appendix 7), but these samples may also be valuable for stress-related studies comparing past and present aspects of biology and population status for these animals.

Geraci suggested that the structure of adrenal gland specimens already examined (Myrick and Perkins, 1995) should be reexamined and compared to newly collected specimens.

Any existing blood samples, including those collected by Scott and colleagues (see IATTC, 1993) should also be reexamined by the same laboratory(s) analyzing samples collected in the necropsy program and repeated chase/capture study. Geraci noted that for comparing blood panels, collection (mode of preservation) and laboratory methods must be taken into account. Most clinical laboratories establish their own ranges of normal values, and it is best to have internal standards and use control samples.

### Summary

Extensive discussions of workshop participants led to the development of three types of biological studies to investigate the potential for fisheries-induced stress affecting dolphins in the ETP. These investigations include: 1) a Necropsy Program to examine tissues from dolphins killed during fishing operations, 2) a Repeated Chase/Capture study designed to analyze blood chemistry of samples collected from live dolphins during a regime of repeated chase and encirclement, and 3) Historical Analysis examining adrenal gland, blood, and potentially, life history specimens and data previously collected from dolphins involved in the tuna purse-seine fishery.

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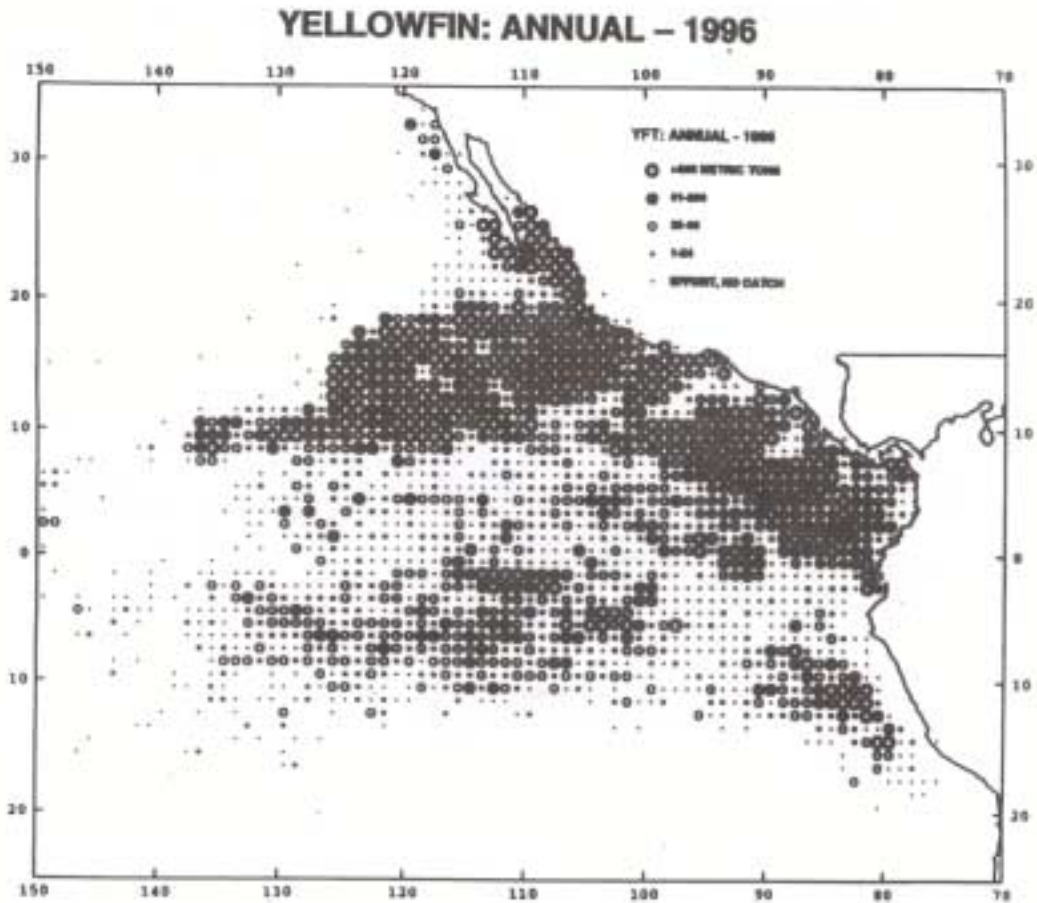


Figure 1. Map showing fishing grounds for yellowfin tuna and fishing effort (metric tons) for 1996 in the eastern tropical Pacific Ocean tuna purse-seine fishery. From IATTC, 1997.

## Biological Responses to Stress: Stimulus, Response, Consequences

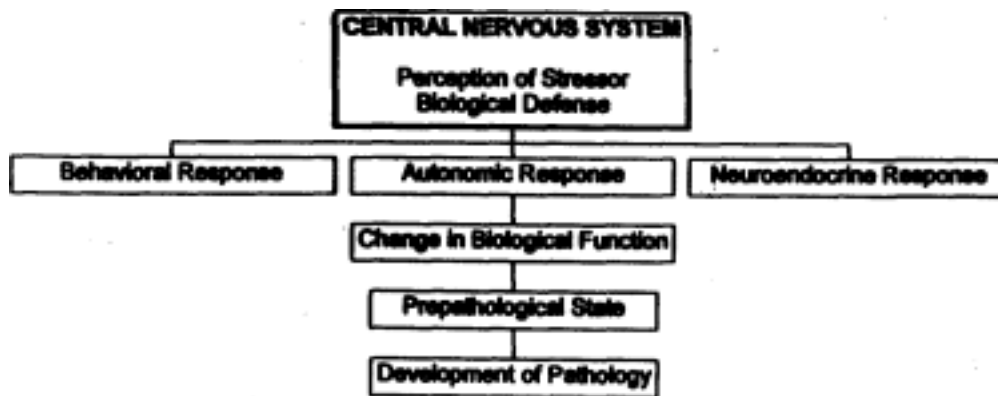


Figure 2. Diagram depicting the perceived stressor and the biological stress response. According to Moberg (1985), the recognition of a threat to homeostasis evokes the stress response and may lead to a prepathological state with the potential for the development of pathology (disease, abnormal growth, loss of reproduction, deleterious behaviors).



Table 1. Reproductive organs (and/or reproductive data), teeth, and skin/blubber samples collected in the ETP tuna purse-seine fishery from 1971-1994 and archived at the SWFSC. Columns represent number of individuals. Similar tissue and/or data are available, in significantly fewer numbers, for seven other cetacean species killed in the fishery.

<b>Species or Stock</b>	<b>Reproductive Organs/Data</b>		<b>Teeth</b>	<b>Skin/Blubber</b>
	<b>Males</b>	<b>Females</b>		
Pantropical spotted dolphin	5903	9447	18,166	459
Eastern spinner dolphin	1899	2027	4633	92
Whitebelly spinner dolphin	1892	2036	4488	58
Common dolphin	971	1033	2095	11

Table 2. Some published hematological and serum chemistry values for the cetacean species most commonly studied in captivity, the bottlenose dolphin (*Tursiops truncatus*). This information was modified from Bossart and Dierauf (1990) who compiled it in an attempt to provide an indication of normal ranges of blood parameters in cetaceans based on data published by Englehardt (1979) and Asper et al. (1990).

Parameter	Unit	Range
<b>Erythrocytes</b>		
<b>N=136</b>		
Red blood cells	$10^6/\text{mm}^3$	2.9-5.4
Hematicrit	%	36.2-51.0
Hemoglobin	g/dl	12.7-18.1
Mean corpuscular volume	fl	101-143
Mean corpuscular hemoglobin	pg	34-50
Mean corpuscular hemoglobin concentration	g/dl	30-38
<b>Leukocytes</b>		
<b>N=152</b>		
White blood cells	$10^3/\text{mm}^3$	4.1-10.8
Neutrophils	%	53-62
Lymphocytes	%	15-28
Monocytes	%	0-8
Basophils	%	0-3
Eosinophils	%	7-37
<b>Electrolytes</b>		
<b>N=80</b>		
Sodium	mEq/l	141-168
Potassium	mEq/l	3.5-5.7
Chloride	mEq/l	100-128
Calcium	mg/dl	7.7-9.7
Phosphorus	mg/dl	4.1-7.8
Iron	ug/dl	110-175 (M) 143-220 (F)
<b>Glucose and lipids</b>		
<b>N=118</b>		
Glucose	mg/dl	87-150
Cholesterol	mg/dl	87-380
Triglycerides	mg/dl	77-157
<b>Liver, muscle, kidney enzymes</b>		
<b>N=111</b>		
Creatine kinase	IU/l	14-486
BUN	mg/dl	31-73
Creatinine	mg/dl	1.1-2.5
Uric acid	mg/dl	0.0-1.8
Alkaline phosphatase	IU/l	123-1050
Lactate dehydrogenase	IU/l	159-1393

Table 2 (continued). Some published hematological and serum chemistry values for the cetacean species most commonly studied in captivity, the bottlenose dolphin (*Tursiops truncatus*).

Parameter	Unit	Range
<b>Liver Enzymes and bilirubin</b>		
<b>N=82</b>		
Total bilirubin	mg/dl	0.0-0.1
Aspartate aminotransferase	IU/l	48-250
Alanine aminotransferase	IU/l	8-47
<b>Proteins</b>		
<b>N=80</b>		
Total protein	g/dl	6.2-8.3
Albumin	g/dl	2.8-5.6
Globulin	g/dl	1.1-3.9
<b>Hormones†</b>		
<b>N=72</b>		
Cortisol*	ug/dl	8.5-24.2
Aldosterone	pg/ml	3-408
Triiodothyronine (T3), total	ng/dl	81-258
Throxine (T4), total	ug/dl	8.5-24.2

† St. Aubin et al., 1996, measured circulating levels of thyroid and adrenal hormones in 72 free-ranging and captive bottlenose dolphins. They compared hormone levels by sex and status, and found significant differences in the following: cortisol (status), aldosterone (status), total T4 (sex, status), total T3 (sex). The authors concluded that differences in levels of adrenal hormones between wild and semi-domesticated dolphins were indicative of a mild stress response.

\* Suzuki et al., 1998 reported levels of circulating cortisol for 15 cetacean species. Radio Immuno Assay (RIA) was used to measure serum cortisol concentrations for 230 captive bottlenose dolphins (ng/ml, 11.0 ± 9.2, r=1.8-20.2).

Table 3a. A list of some primary modes of investigation and indicators of physiological stress or overall health and disease status for post-mortem specimens of dolphins killed in the tuna purse-seine fishery. The list illustrates types of samples to be collected in the necropsy program. For all animals possible, a complete necropsy will be performed providing detailed descriptions of any grossly evident lesions, weighing all organs, and removing representative tissues from all organ systems. Sample collection will include a variety of preservation methods dependent upon the tissue and analyses required. Selected references are listed in some cases, however Howard (1983a&b), Dierauf (1990a), Geraci and Lounsbury (1993), and Jones *et al.* (1997), have also reviewed relevant aspects of veterinary pathology, cetacean health and disease, and sample collection.

<b>Investigation</b>	<b>Sample</b>	<b>Indicator</b>	<b>Selected References</b>
Liver/pancreatic function	1,2,3	hypoperfusion; elevated blood enzyme levels	Geraci and St.Aubin, 1979
Thyroid gland morphology/function	1,2,3	enlargement; changes in concentrations of T3,T4	Ridgway and Patton,1971; St. Aubin and Geraci, 1988; 1992
Adrenal gland morphology/function	1,2,3	tissue damage; inflammation; enlargement; elevated blood cortisol, aldosterone	Junquera and Carneiro, 1980; Harvey <i>et al.</i> , 1984
Heart condition	1,2,3	ischemic injuries; myocardial necrosis	Cowan and Walker, 1979; Cowan <i>et al.</i> , 1986; Cebelin and Hirsch, 1980
Muscle condition/function	1,2,3	evidence of myolysis, degradation; fiber typing; pH, lactic acid levels	Close, 1972; Goforth, 1984 Bateson and Bradshaw, 1997

Legend to Tables 3a and b:

1 = entire organ/gland/system (as required)	4 = blubber/skin	7 = brain/kidney/liver
2 = organ (tissue) sections	5 = swabs of body fluids/mucous	8 = teeth
3 = blood	6 = parasite	

Table 3b. A list of some primary modes of investigation and indicators of overall health and disease status (and potential indicators of physiological stress) for post-mortem specimens of dolphins killed in the tuna purse-seine fishery.

Investigation	Sample	Selected References
Microbiology/Virology	5	Geraci and Lounsbury, 1993; Carter and Cole, 1990
Reproduction/Life History	1,2,3,8	Hohn <i>et al.</i> , 1985; Chivers and Myrick, 1992; Chivers and Demaster, 1994; Myrick <i>et al.</i> , 1983
Parasitology	1,2,6	Dailey and Perrin, 1973; Walker and and Cowan, 1981
Body and nutritional condition	4,2	Lockyer, 1987 a, b; see Worthy and Edwards, 1990
Respiratory condition	1,2	
Gastrointestinal tract condition	1,2	Gaskin, 1978
Kidney condition/function	1,2,3	
Splenic condition	1,2	Cowan and Walker, 1979
Brain condition	1,2	
Toxicology	4,7	Aguilar and Borrell, 1997; O'Shea <i>et al.</i> , 1980
Immunology	2,3	Keller <i>et al.</i> , 1991; Romano <i>et al.</i> , 1992

Legend to Tables 3a and b:

1 = entire organ/gland/system (as required)	4 = blubber/skin	7 = brain/kidney/liver
2 = organ (tissue) sections	5 = swabs of body fluids/mucous	8 = teeth
3 = blood	6 = parasite	

Table 4. Some predicted changes, over time after repeated chase and recapture, in values from blood and other tissue analyses of ETP dolphins. Changes noted are given in relation to predicted values listed in the previous column. These are theoretical values; actual values would be dependent upon length and intensity of chase, and severity of exertion and psychological affects. These values were presented by Geraci during discussions of the potential for measuring the effects of chase and capture over time. Indications of cumulative effects, if present, were suggested to be most readily apparent on day four.

<b>BLOOD LEVELS:</b>	<b>TIME:</b>			
	<b>&lt; 6 hours</b>	<b>1 day</b>	<b>2 days*</b>	<b>4 days</b>
<b>neutrophils</b>	increased	similar values	increased	similar or increasing values
<b>eosinophils</b>	decreased	significantly decreased	decreased	similar or decreasing values
<b>lymphocytes</b>	no effect or slightly decreased	decreased	decreased	similar or decreasing values
<b>iron</b>	no affect or slightly decreased	decreased	similar or decreasing values	similar or decreasing values
<b>cortisol/ hydrocortisone</b>	significantly increased; possibly returned to normal	returned to normal	no interaction	no interaction
<b>epinephrine</b>	significantly increased; possibly returned to normal	no interaction	no interaction	no interaction
<b>muscle enzymes</b>	increased	significantly increased	significantly increased	similar or increasing values

Table 4 (continued). Some predicted changes, over time after repeated chase and recapture, in values from blood and other tissue analyses of ETP dolphins.

<b>TIME:</b>				
	<b>&lt; 6 hours</b>	<b>1 day</b>	<b>2 days*</b>	<b>4 days</b>
<b>HISTOLOGICAL EVIDENCE:</b>				
<b>muscle</b>	no perceptible effect	perceptible effect	perceptible effect	perceptible effect
<b>heart</b>	possible perceptible effect	perceptible effect	perceptible effect	perceptible effect

\* Possible to have no further observed effects on day two. Conditions listed are those that would result to damage to heart muscle or other potential lesions.

## APPENDIX 1

### LIST OF PARTICIPANTS

#### NMFS WORKSHOP TO INVESTIGATE THE POTENTIAL INFLUENCE OF FISHERY-INDUCED STRESS ON ETP DOLPHINS

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## APPENDIX 2

### REVISED AGENDA

#### NMFS WORKSHOP TO INVESTIGATE THE INFLUENCE OF FISHERY-INDUCED STRESS ON ETP DOLPHINS

Southwest Fisheries Science Center  
La Jolla

Thursday, July 10, 1997 (8:30 a.m.-5:00 p.m.)

##### I. INTRODUCTION

- Who we are, why we are here, what we will do, product anticipated

##### II. INFORMATION PRESENTATION

- ETP Fishery/Dolphin Information (Edwards)
- ETP Dolphin Population Dynamics (Gerrodette)
- Practical Aspects of Sample Collection (Scott)
- Meaning and Measurement of Stress (Cowan)
- Physiological Responses to Stress in Dolphins (Geraci)
- Corticosteroid Detection in Cetacean Blood and Blubber Samples  
(Palmer)
- Physiological Responses to Stress in Domestic and Other Large Mammals  
(Friend)

Friday, July 11, 1997 (8:30 a.m.-5:00 p.m.)

##### III. QUESTIONS LIST

- Stressors and responses
- Factors affecting stress responses
- Sampling (measuring) stress responses
- Previous/proposed work

##### IV. WORKSHOP SUMMARY

## APPENDIX 3

### SWFSC REPORTS RELATED TO STRESS IN ETP DOLPHINS

- Cowan, D.F. and W.A. Walker. 1979. Disease factors in *Stenella attenuata* and *S. longirostris* taken in the eastern tropical Pacific yellowfin tuna purse-seine fishery. Southwest Fisheries Center Admin. Rept. LJ-79-32C. 20 p.
- Stunz, W.E. and T.B. Shay. 1979. Report on capture stress workshop. Southwest Fisheries Center Admin. Rept. LJ-79-28. 24 p.
- Coe, J.M. and W.E. Stunz. 1980. Passive Behavior by the spotted dolphin, *Stenella attenuata*, in tuna purse seine nets. Fish. Bull. 78(2): 535-537.
- Myrick, A. 1995. Documentation of progress of stress research projects for future reference. Southwest Fisheries Science Center Admin. Rept. LJ-95-01. 14 p.
- Myrick, A. and P.C. Perkins. 1995. Adrenocortical color darkness and correlates as indicators of continuous acute premortem stress in chased and purse-seine captured male dolphins. Pathophysiology 2:191-204. 14 p.
- Perkins, P.C. and E.F. Edwards. 1997. Capture rate as a function of school size in offshore spotter dolphins in the eastern tropical Pacific Ocean. Southwest Fisheries Science Center Admin. Rept. LJ-97-03. 36 p.

## APPENDIX 4

### PHYSIOLOGICAL MEASUREMENTS QUESTIONS LIST

#### I. Stressors and Expected Responses with respect to Dolphin Sets

##### A. During Sets

1. During sighting
2. During chase
3. During encirclement
4. During confinement (pre-backdown)
5. During backdown (waiting)
6. During backdown (un-aided spillout over corkline)
7. During backdown (crew-aided spillout)

##### B. Non-Set Periods

1. When no vessels are around (i.e., "normal" life in the ETP)
2. Set-related (i.e., "residual" stress effects due to fishery)
3. Recovery (short term/long-lasting effects)
4. Recovery (time for each kind of stress response)

## II. Factors Affecting Stress Responses

### A. Dolphin Sets (Habituating/Sensitizing Type of Stress)

### B. Dolphin Characteristics

Individual personality (within age/sex/species grouping): What fraction of the population is likely to have stress effects strong enough to affect reproductive success and/or survival?

1. Individual history/personality

2. Stock-specific/species-specific personality

3. Size

4. Age

5. Sex

6. Other

7. Fishing history (capture frequency): ETP dolphins are caught on average 8 times per year. Realistically, most are probably caught fewer times (as little as 1-2 times per year, if at all), while a few may be caught as frequently as 40-50 times per year. What sorts of stress effects might one expect to see with capture frequencies of this order, both in the short term and over a life span?

8. Life span: Dolphins in the ETP fishery today must have grown up with the practice of dolphin fishing, because that method was introduced in about 1960, 37 years ago. What are the likely effects on stress measurements (e.g. what to measure)?

## II. Sampling (Measuring) Stress Responses

What can we sample?

What will those samples tell us?

What won't those samples tell us?

### A. Sampling Constraints

1. Research vessels
2. Non-chartered seiner
3. Chartered seiner
4. Fishery location and seasonality (remote, slightly seasonal)
5. Non-US composition of fleet

### B. Controls

What is acceptable/necessary?

Are other species/places acceptable?

### C. Population Level Stressors (affecting survival/reproduction)

What can be measured in the ETP?

### D. Individual Stressors (affecting a single animal, short or long term)

What can be measured in the ETP?

**MARINE MAMMAL SIGHTING  
AND SET RECORD**

DATE                   CRUISE NO.                   TIME                   MARINE MAMMALS SIGHTED                   SIGHTING NO.                   SET NO.                   PAGE 1

**1. INITIAL ESTIMATE OF NUMBER AND SPECIES COMPOSITION OF ENTIRE SCHOOL**

	TOTAL NUMBER	% SPOTTED	% E-SPIN	% WS-SPIN	% UNIDSPIN	% COMMON	% OTHER SPECIES (1)	% OTHER SPECIES (2)	SPOTTED STOCK	OTHER SPECIES/ STOCK (1)	OTHER SPECIES/ STOCK (2)
TECHNICIAN	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>
CREW	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>
AERIAL	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>	<u>  </u>
									CODE	CODE	CODE

**2. SIGHTING AND IDENTIFICATION NOTES**

SIGHTING NOTES: \_\_\_\_\_  
LIST ALL CHARACTERISTICS USED FOR IDENTIFICATION AND MAKE A DRAWING OF EACH SPECIES OBSERVED

FOR SPOTTED	
NEO	<u>  </u> %
2-TONE	<u>  </u> %
SPECK	<u>  </u> %
MOTT	<u>  </u> %
FUSED	<u>  </u> %

CREW'S ESTIMATE	
TOTAL NO.	% COMPOSITION BY SPECIES
1) <u>  </u>	<u>  </u>
2) <u>  </u>	<u>  </u>
3) <u>  </u>	<u>  </u>

INDICATE CLOSEST DISTANCE YOU OBSERVED EACH SPECIES   

DATA REVIEWED ☐

NUMBER 11/88

APPENDIX 5





CRUISE NO. \_\_\_\_\_ SET NO. \_\_\_\_\_

PAGE 3

6. ESTIMATE OF NUMBER AND SPECIES COMPOSITION OF CAPTURED MARINE MAMMALS

6. ESTIMATE OF NUMBER AND SPECIES COMPOSITION OF CAPTURED MARINE MAMMALS								SPOTTED STOCK	OTHER SPECIES 1	OTHER SPECIES 2
TOTAL NUMBER	% SPOTTED	% E-SPIN	% WB-SPIN	% UNIDSPIN	% COMMON	% OTHER (1)	% OTHER (2)			
TECHNICIAN _____								_____	_____	_____
CREW _____								_____	_____	_____
TOTAL % COMPOSITION BY SPECIES								CODE	CODE	CODE
CREW'S ESTIMATE 1.) _____								NEONATES CAPTURED? YES ____ NO ____		
2.) _____										
3.) _____										

7. LIVE MARINE MAMMALS RELEASED AFTER RINGS UP

(a.) PRIOR TO BACKDOWN (IF NO BACKDOWN GO TO ITEM 1d.)				(b.) BY BACKING DOWN		(c.) DURING BACKDOWN (DO NOT INCLUDE ANIMALS BACKED OUT)				NO. OF LIVE MARINE MAMMALS LEFT IN NET AFTER BACKDOWN	(d.) FOLLOWING BACKDOWN TO BEGINNING OF SACKUP OR IF NO BACKDOWN					(e.) SACK RESCUE	
SUB T	R A T	S W I M	NO. ACTIVELY RELEASED	NO. BACKED OUT	TONS FISH LOST	SUB T	R A T	S W I M	NO. ACTIVELY RELEASED		SUB T	R A T	S W I M	DRUG	CH-TIME	NO. ACTIVELY RELEASED	NO. ACTIVELY RELEASED
_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____

8. DESCRIBE ALL RESCUE EFFORTS AND BACKDOWN PROCEDURE (INCLUDE TIMES):

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

9. BEST ESTIMATE OF NUMBER AND SPECIES COMPOSITION OF ENTIRE SCHOOL

9. BEST ESTIMATE OF NUMBER AND SPECIES COMPOSITION OF ENTIRE SCHOOL								SPOTTED STOCK	OTHER SPECIES 1	OTHER SPECIES 2
TOTAL NUMBER	% SPOTTED	% E-SPIN	% WB-SPIN	% UNIDSPIN	% COMMON	% OTHER (1)	% OTHER (2)			
TECHNICIAN _____								CODE	CODE	CODE
DATA REVIEWED <input type="checkbox"/>										

PAGE 4

NOTE: AN "INJURED" DOLPHIN IS ONE THAT APPEARS TO HAVE BROKEN BONES, FLIPPERS, OR SNOUT, OR SEVERELY TORN FLIPPERS, FINS, OR FLUKES. ANIMALS THAT ARE BLEEDING PROFUSELY ARE TO BE CONSIDERED AS INJURED, BUT DO NOT CONFUSE PROFUSE BLEEDING WITH SUPERFICIAL NET ABRASIONS. ANIMALS ROLLED THROUGH THE POWER BLOCK ARE TALLIED AS DEAD.

© = INJURED

7 - STATUS UNDETERMINED

[illegible]DATA REVIEWED ☐

## 10. MARINE MAMMAL MORTALITY AND PHYSICAL CAUSES

SPECIES/STOCK KILLED, INJURED OR UNDETERMINED	TOTAL NUMBER KILLED	PERCENTAGE KILL ACCORDING TO CAUSE			NUMBER KILLED			NUMBER INJURED			UNDETERMINED STATUS
		TANGLED OR ENTRAPPED	DIED IN SACK	OTHER	M	F	UNK SEX	M	F	UNK SEX	
SPOTTED											
SPINNER											
OTHER SP/STK.											
OTHER SP/STK.											
OTHER SP/STK.											

DETAIL SPOTTED MORTALITY BY COLOR PATTERN/AGE AND SEX															
NEONATE			TWO-TONE			SPECKLED			MOTTLED			ADULT			AGE UNK
M	F	UNK SEX	M	F	UNK SEX	M	F	UNK SEX	M	F	UNK SEX	M	F	UNK SEX	

SKETCH DORSAL FIN, VENTRAL  
HUMP AND COLOR PATTERN OF  
EACH SPECIES/STOCK KILLED IN  
SET



SP/STK #1 \_\_\_\_\_



SP/STK #2 \_\_\_\_\_



SP/STK #3 \_\_\_\_\_

DESCRIBE CAUSES OF MORTALITY, INJURY, AND STATUS UNDETERMINED \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

CRUISE NO. \_\_\_\_\_ SET NO. \_\_\_\_\_

PAGE 9

- 11.) INDICATE THE NUMBER OF BOW BUNCHES PULLED DURING THIS SET. . . . . \_\_\_\_\_
- 12.) INDICATE THE NUMBER OF SPEEDBOATS USED TO TOW ON THE NET. . . . . \_\_\_\_\_
- 13.) DURING BACKDOWN WAS THE PERIMETER OF THE BACKDOWN CHANNEL COVERED BY THE SAFETY PANEL SYSTEM? . . . . . \_\_\_\_\_  
(DRAW BOUNDARIES OF THE SAFETY PANEL SYSTEM IN YOUR BACKDOWN ILLUSTRATIONS ON PAGES 7 & 8 - REFER TO MANUAL FOR PROPER RESPONSE TO THIS QUESTION)
- 14.) WAS A STRONG CURRENT EVIDENT THIS SET? . . . . . \_\_\_\_\_  
a.) HOW DID YOU DETERMINE THAT THERE WAS A STRONG CURRENT? \_\_\_\_\_
- 15.) DID A NET COLLAPSE INVOLVING MARINE MAMMALS OCCUR AT ANY TIME DURING THIS SET? . . . . . \_\_\_\_\_  
a.) WERE MAMMALS KILLED AS A RESULT OF NET COLLAPSE? . . . . . \_\_\_\_\_  
b.) HOW WAS THE COLLAPSE CLEARED? \_\_\_\_\_
- 16.) WERE CANOPIES EVIDENT DURING THIS SET? . . . . . \_\_\_\_\_  
a.) WERE MARINE MAMMALS KILLED AS A RESULT OF A CANOPY? . . . . . \_\_\_\_\_  
b.) HOW WAS THE CANOPY CLEARED? \_\_\_\_\_
- 17.) IF ALL OR PART OF BACKDOWN OCCURRED IN THE DARK, WERE LIGHTS USED BY THE VESSEL TO ILLUMINATE THE BACKDOWN CHANNEL? (HIF = HIGH INTENSITY FLOODLIGHT, Y = OTHER LIGHTS, N = NO LIGHTS, N/A = NOT DARK) . . . . . \_\_\_\_\_  
a.) DESCRIBE THEIR TYPE, LOCATION, AND USE \_\_\_\_\_






- 18.) DID A MALFUNCTION OCCUR DURING THIS SET? . . . . . \_\_\_\_\_  
a.) DESCRIBE ALL MALFUNCTIONS IN ORDER OF OCCURRENCE.

	TIME OCCURRED	TIME FIXED	DELAY IN SET	(DESCRIBE TYPE OF MALFUNCTION, OPINIONS OF CAUSE AND HOW FIXED)	WERE LIVE MARINE MAMMALS IN NET?	MALF. CODE
1						
2						
3						
4						

DATA REVIEWED ☐



## 19. NET CONFIGURATIONS, SET ILLUSTRATIONS (CONTINUE ILLUSTRATIONS UNTIL ALL LIVE DOLPHINS ARE OUT OF NET)

SYMBOLS			IF YOU USE OTHER SYMBOLS DRAW AND DEFINE HERE:
X DOLPHINS	⊙ BOW BLANCH	 SPEEDBOAT TOWING ON CORKLINE	
E ENTANGLEMENT/ENTRAPMENT	⊗ RAFT	 VESSEL	
W WIND DIRECTION	 SKIFF	 DOLPHIN SAFETY PANEL LOCATION	
D CURRENT DIRECTION	 SPEEDBOAT		
C CANOPY			
S CORK SINKAGE			

TIME	TIME	TIME
		

NOTES:	NOTES:	NOTES:

TIME	TIME	TIME
		

NOTES:	NOTES:	NOTES:

CRUISE NO.

SET NO.

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## 19. NET CONFIGURATIONS (CONTINUED)

TIME	TIME	TIME
<div></div>	<div></div>	<div></div>
NOTES:	NOTES:	NOTES:

TIME	TIME	TIME
<div></div>	<div></div>	<div></div>
NOTES:	NOTES:	NOTES:

## 20. COMMENTS BY THE CAPTAIN REGARDING THIS SET:





## APPENDIX 6

### Corticosteroid Detection in Cetacean Blood and Blubber Samples

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Analysis of stress in cetaceans by determining corticosteroid levels in small biopsy samples is conceivable. Corticosteroids are involved in the complex stress response of all vertebrates to both osmotic (mineralocorticoid-mediated) and stress (glucocorticoid-mediated) challenges. Since the evolutionary history of cetaceans includes the unique osmotic challenge of evolution back to the marine environment, and different families of vertebrates utilize different major corticosteroids in the stress response, there was motivation to develop a method which would detect all corticosteroids in a single assay.

The separation of all vertebrate corticosteroids (21-hydroxylated 4-pregen series): Aldosterone, 18-OH-corticosterone, 18-OH-11-deoxycorticosterone, corticosterone, 11-deoxycorticosterone, cortisol, 11-deoxycortisol, and their immediate precursors, 17-hydroxyprogesterone and progesterone by a mode of capillary electrophoresis known as electrokinetic chromatography (EKC) was achieved. This assay is complete in less than twenty minutes and has a biological limit of detection of less than 1 ng/ml using concentrated samples. In addition, the technique separates corticosteroids according to gross structure, allowing the potential for detection of novel corticosteroids

Precise extraction of all corticosteroids from complex sample matrices such as blubber requires a discerning method, as both corticosteroids and potential lipid interferences span a wide range of polarities. A preliminary solvent-extraction gives recoveries between 50-90% for authentic steroids spiked in clean serum samples. The lipid content in Mysticeti samples (blood or blubber) makes them more challenging to extract than Odontoceti. A methanol precipitation is necessary for analysis of high lipid serum, blood lysate, and blubber samples. Recoveries for authentic steroids spiked into blubber samples with the methanol precipitation step are more variable, probably due to compartmentalization of the steroids in the extracted lipids.

Extracted *Tursiops truncatus* serum indicates cortisol was predominant in the animal sampled, with the presence of aldosterone and an unidentified compound with electrophoretic characteristics like 11-deoxycorticosterone and 17-hydroxyprogesterone. *Megaptera novaeangliae*, *Balaena rriysticetus*, *Delphinapterus leucas* and other samples are compared. Validation and precise quantification requires optimization of the extraction process and analysis of samples from stressed and unstressed cetaceans. This technique may be developed into a small-volume biopsy (100ul) analysis to provide insight into corticosteroid levels of wild cetaceans.

## APPENDIX 7

### SOME PUBLICATIONS RESULTING FROM BIOLOGICAL DATA COLLECTED FROM DOLPHINS INCIDENTALLY KILLED IN THE TUNA/DOLPHIN PURSE-SEINE FISHERY

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In addition, the Report of the International Whaling Commission contains numerous reports on the composition of kill in the ETP tuna purse-seine fishery with length frequencies and life history information (reproductive condition for females of various species). A number of abstracts from the Status of Porpoise Stocks (SOPS) Workshop, 18-22 November 1991 held in La Jolla, CA by the SWFSC, contain biological information from dolphins killed in the fishery.

